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Reconstructing Austronesian population history in Island Southeast Asia

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Abstract

Austronesian languages are spread across half the globe, from Easter Island to Madagascar. Evidence from linguistics and archaeology indicates that the “Austronesian expansion,” which began 4–5 thousand years ago, likely had roots in Taiwan, but the ancestry of present-day Austronesian-speaking populations remains controversial. Here, focusing primarily on Island Southeast Asia, we analyze genome-wide data from 56 populations using new methods for tracing ancestral gene flow. We show that all sampled Austronesian groups harbor ancestry that is more closely related to aboriginal Taiwanese than to any present-day mainland population. Surprisingly, western Island Southeast Asian populations have also inherited ancestry from a source nested within the variation of present-day populations speaking Austro-Asiatic languages, which have historically been nearly exclusive to the mainland. Thus, either there was once a substantial Austro-Asiatic presence in Island Southeast Asia, or Austronesian speakers migrated to and through the mainland, admixing there before continuing to western Indonesia.

The history of the Austronesian (AN) expansion and of populations speaking AN languages has long been of interest. Patterns of lexical diversity within the AN language family point to Taiwan as the AN homeland (1, 2), as do elements of the archaeological record, for example red-slipped pottery and Taiwanese-mined nephrite (3–5). However, some authors have argued that the AN expansion was driven primarily by cultural diffusion rather than large-scale migration (6–8), and other associated artifacts, such as cord-marked and circle-stamped pottery, likely derive instead from the mainland (9, 10). It is also unknown how the history of populations in western Island Southeast Asia (ISEA), which speak Western Malayo-Polynesian AN languages, differs from that of Central and Eastern Malayo-Polynesian speakers in eastern Indonesia and Oceania.

Genetic data can be used to trace human migrations and interactions in a way that is complementary to the information provided by linguistics and archaeology. Some single-locus genetic

studies have found affinities between Oceanian populations and aboriginal Taiwanese (11–15), but others have proposed that present-day AN speakers do not have significant genetic inheritance from Taiwan (16–18). Within Indonesia, several surveys have noted an east–west genetic divide, with western populations tracing a substantial proportion of their ancestry to a source that diverged from Taiwanese lineages 10–30 thousand years ago (kya), which has been hypothesized to reflect a pre-Neolithic migration from Mainland Southeast Asia (MSEA) (19–22). Genome-wide studies of AN-speaking populations, which in principle can provide greater resolution, have been interpreted as supporting both Taiwan-centered (23, 24) and multiple-wave (21) models. However, such work has relied primarily on clustering methods and fitting bifurcating trees that do not model historical admixture events, even though it is well known that many AN-speaking populations are admixed (21, 24–28). Thus, these studies have not established firmly whether AN speakers have ancestry that is descended from Taiwan, MSEA, or both.

Here, we explore these questions by reconstructing the genome-wide ancestry of a diverse sample of AN-speaking populations, predominantly within ISEA. We apply novel methods for determining the phylogenetic placement of sources of gene flow in admixed populations and identify four major ancestry components, including one linked to Taiwan and a second Asian component from MSEA.

Results

Analysis of admixed populations

To investigate the ancestry of AN-speaking populations at high resolution, we analyzed a genome-wide data set of 31 AN-speaking and 25 other groups from the HUGO Pan-Asian SNP Consortium (25) and the CEPH-Human Genome Diversity Panel (HGDP) (29). We used genotypes

from 18,412 single nucleotide polymorphisms (SNPs) that overlapped across all samples (see Methods, Supplementary Table 1, and Supplementary Fig. 1). To confirm that our results are robust to the way SNPs were chosen, we repeated our primary analyses with data obtained by merging the Pan-Asia genotypes with HGDP samples typed on the Affymetrix Human Origins array (30) (see Methods and Supplementary Tables 6 and 7). For some tests requiring denser markers, we also used a smaller set of 10 AN-speaking groups first published in ref. (27) and typed at over 500,000 SNPs.

We developed new methods to analyze the data, which we release here as the *MixMapper* 2.0 software. *MixMapper* is a tool for building phylogenetic models of population relationships that incorporate the possibility of admixture. Both the original version (31) and *MixMapper* 2.0 use allele frequency correlations to construct an unadmixed scaffold tree and then add admixed populations. The entire best-fitting model for each admixed population, including mixture proportions and the placement of the sources of ancestry on the scaffold, is inferred from the data, and uncertainty in parameter estimates is measured through bootstrap resampling (see Methods). *MixMapper* 2.0 substantially improves the three-way mixture fitting procedure of the original program, as it implements a rigorous test to determine whether populations are best modeled via two- or three-way admixtures. It also allows for full optimization of the inferred mixture proportions (see Methods). A strength of *MixMapper* and related methods is that the underlying allele frequency correlation statistics, and hence the inferences about population relationships, are largely robust to the way that SNPs are chosen for analysis (30–32).

We selected a scaffold tree consisting of 18 populations that are approximately unadmixed relative to each other (Fig. 1; Supplementary Tables 2 and 3): Ami and Atayal (aboriginal Taiwanese); Miao, She, Jiamao, Lahu, Wa, Yi, and Naxi (Chinese); Hmong, Plang, H'tin, and Palaung (from Thailand); Karitiana and Suruí (South Americans); Papuan (from New Guinea); and Mandenka and Yoruba (Africans). This set was designed to include a diverse geographical

and linguistic sampling of Southeast Asia (in particular Thailand and southern China) along with outgroups from other continents, which are necessary for accurate mixture fitting (31) (see Methods). We have previously shown that *MixMapper* results are robust to the choice of scaffold populations (31), and indeed our findings here were essentially unchanged when we repeated our analyses with an alternative, 15-population scaffold (Supplementary Fig. 2; Supplementary Tables 8 and 9) and with 17 perturbed versions of the original scaffold (Supplementary Tables 10 and 11). Using this scaffold tree, we obtained confident results for 25 AN-speaking populations (for geographical locations, see Fig. 2): eight from the Philippines, nine from eastern Indonesia and Oceania, and eight from western ISEA. Several populations in our data set—Batak Karo, Ilocano, Malay, Malay Minangkabau, Mentawai, and Temuan—were not as readily fit with *MixMapper*, which we hypothesize was due to the presence of additional ancestry components that we could not capture well in our modeling framework. Thus, we omit these populations from further analyses, although we note that their *MixMapper* results, while not as reliable, were still similar to those for the 25 groups discussed here.

All admixed AN-speaking populations fit best as combinations of two or three ancestry components out of a set of four: one closely related to Papuans (“Melanesian”), one splitting deeply from the Papuan branch (“Negrito”), one most closely related to aboriginal Taiwanese, and one most closely related to H’tin (Fig. 1). While the relative proportions varied substantially from group to group, the (independently inferred) positions of the ancestral mixing populations were highly consistent, leading us to assign them to these four discrete sources (Fig. 1). A total of 14 populations were best modeled as two-way admixed (Supplementary Table 4): all eight from the Philippines (with Taiwan-related and Negrito ancestry), four from eastern Indonesia (with Taiwan-related and Melanesian ancestry), and both from Oceania (Fiji and Polynesia, merged from ref. (27); also Taiwan-related and Melanesian). The remaining 11 populations, including all eight from western ISEA, fit best as three-way admixed (Supplementary Table 5),

with both Taiwan-related and H'tin-related ancestry (Supplementary Table 12). Among the 25 groups, the Taiwan-related component was inferred to account for approximately 30–90% of ancestry, while for the 11 three-way admixed groups, the H'tin-related component was inferred to account for approximately 10–60%. By contrast, we found no Taiwan-related ancestry in admixed MSEA populations speaking non-AN languages (Fig. 2; Supplementary Table 13). We note that our estimates of mixture proportions are robust to alternative histories involving multiple waves of admixture or continuous migration, since *MixMapper* is based on allele-sharing statistics that measure the probability of descent from each possible source of ancestry. Thus, continuous gene flow scenarios that preserve the same topology relating the admixed population to the scaffold tree will produce the same estimates of mixture proportions (30,31).

To obtain an independent estimate of how many sources of admixture are necessary to explain the observed relationships among populations from ISEA, we applied a formal test (33,34) that analyzes f_4 statistics among a set of admixed and outgroup populations to determine a lower bound on the total number of ancestry sources (Supplementary Table 14). For the Philippines, we found that a maximal subset of six groups (Agta, Ati, Ayta, Ilocano, Iraya, and Manobo) could be consistently modeled as derived from a single pair of mixing populations (Supplementary Fig. 1A). Likewise, the four eastern Indonesian groups (Alorese, Kambara, Lamaholot, and Lembata) that were inferred to be two-way admixed by *MixMapper* could be modeled with two total ancestry sources according to the f_4 -based test (Supplementary Fig. 1B). However, adding the two Manggarai populations required a third source of ancestry, consistent with the H'tin-related ancestry inferred by *MixMapper*. In western ISEA, a large subset of six groups (Bidayuh, Dayak, Javanese Jakarta, Javanese Java, Mentawai, and Sunda) was consistent with being derived from three ancestral mixing populations (Supplementary Fig. 1C), and moderately diverged subsets with as few as three populations (Bidayuh, Dayak, and either Javanese or Sunda) still required three sources of ancestry. Larger subsets were always of greater com-

plexity, indicating some additional, more localized gene flow, such as a likely influx of Indian ancestry in some populations (20, 25). However, the presence of the subsets that can be fit as mixtures of two or three sources increases our confidence that the *MixMapper* models are close to the true history.

Finally, we used our recently developed ALDER software (35) to estimate dates of admixture using linkage disequilibrium. For populations from the Philippines, eastern Indonesia, and Oceania from ref. (27), we obtained dates of 30–65 generations ago assuming a single-pulse model of admixture (0.9–1.8 kya assuming 29 years per generation (36); Supplementary Fig. 3). These dates are considerably more recent than the initial AN expansion as documented through archaeology (2–5), and thus they must reflect additional waves of interaction involving populations with different proportions of Asian ancestry after the initial AN settlement of the islands. We also applied *ALDER* to a merged set of populations from western ISEA and estimated that their admixture occurred 76 ± 21 generations ago (2.2 ± 0.6 kya; Supplementary Fig. 4). Again, this date implies the most recent possible time for the onset of population mixing and should not be interpreted as an estimate of the date of the earliest episodes of admixture (35).

Details of inferred ancestry components

Our results indicate that there is a component of ancestry that is universal among and unique to AN speakers and that always accounts for at least a quarter of their genetic material. This component, moreover, is more closely related to aboriginal Taiwanese than to any population from the mainland. In theory, this ancestry could have been derived from a mainland source that was related to the ancestors of aboriginal Taiwanese but was either displaced by subsequent migrations (such as the expansion of Han Chinese) or whose descendants are not included in our data set. Given our dense sampling of East and Southeast Asian populations, this scenario seems unlikely, but we are unable to formally rule it out.

We also considered the possibility that the direction of flow for this “Austronesian” ancestry component could have been reversed, with an origin in Indonesia or the Philippines and a northward spread to Taiwan. Because of migrations, it is impossible to determine with certainty where ancestral populations lived based on present-day samples, but the fact that the aboriginal Taiwanese populations in our data set, Ami and Atayal, are unadmixed (to within the limits of our resolution), whereas the AN component appears in admixed form in all other AN-speaking populations from ISEA, can be most parsimoniously explained by a Taiwan-to-ISEA direction of gene flow. We verified that Ami and Atayal have no detectable signature of admixture both by the three-population test (30,37) (Supplementary Table 3) and by testing them as putatively admixed in *MixMapper* with a scaffold tree made up of the other 16 original scaffold populations. In the latter analysis, we found that both Ami and Atayal returned best-fitting positions that indicated that they are properly modeled as unadmixed, adjacent to Jiamao (Supplementary Table 15). On the other hand, all other AN-speaking populations, including those with no signal of admixture from the three-population test, continued to fit robustly as admixed on this reduced scaffold, with the AN component now closest to Jiamao, as expected (Supplementary Table 15). Thus, the absence of admixture in Ami and Atayal allows us to conclude that they have a qualitatively different history from other AN-speaking populations in ISEA and that our inferred directionality of gene flow, with Taiwan as the source, is more parsimonious and a better fit to the data.

The second and third ancestry components we infer for AN-speaking populations are Melanesian and Negrito. All admixed groups we tested contain at least one of these components, which we believe reflect admixture with indigenous populations in ISEA. The Melanesian component is closely related to Papuans and is found in the highest proportions among our study populations in easternmost Indonesia and in Fiji (Fig. 2). The Negrito component, meanwhile, forms a deep clade with Papuans and is found in populations from the Philippines and western ISEA

(Fig. 2). We treat this ancestry as deriving from a single ancient source because it clusters phylogenetically across admixed populations, with the branching positions from the scaffold tree inferred to be very similar (Fig. 1B). We use the name “Negrito” to describe this ancestry based on the fact that it occurs in the greatest proportion in Philippine Negrito populations. The Negrito ancestry in western ISEA could be a result of admixture with aboriginal peoples living on these islands or alternatively of prior admixture in the Philippines or on the mainland. We note that with *MixMapper*, we are unable to determine the precise branching position of this component in three-way admixed populations (see Methods), which would in principle shed light on this question. We are also unable to rule out a small proportion of Negrito ancestry in eastern Indonesia and Oceania—which might be plausible if AN speakers migrated from Taiwan through the Philippines first and admixed at that time with indigenous peoples—or a small proportion of Melanesian ancestry in the Philippines, but the large genetic drift separating the branching positions of the two components (Melanesian and Negrito) provides strong evidence that they reflect at least two ancestral sources (Fig. 1).

An unanticipated finding from our study is that populations in western ISEA, as well as a few in eastern Indonesia, also contain an unambiguous signal of an additional source of Asian ancestry, which is assigned with high confidence to an ancestral population splitting roughly two-fifths of the way down the H’tin branch in our scaffold tree (Fig. 1D). The H’tin speak a language belonging to the Austro-Asiatic (AA) family, which is hypothesized to have been the major language group in MSEA following the expansion of rice farming (5). Later dispersals have resulted in substantial replacements of AA languages outside of Cambodia and Vietnam, but AA-speaking tribal groups are still present in areas where Tai, Hmong, and Indo-European languages now predominate, extending as far west as India (5). By contrast, no pockets of AA languages are found at all in present-day ISEA (with the exception of the Nicobar Islands in the Indian Ocean), which, in conjunction with the absence of clear archaeological evidence

of previous settlement by agriculturalists who were not part of the AN cultural complex (10), makes it unlikely that AA-speaking populations previously lived in the areas where we detect AA-related ancestry.

To test the alternative explanation that the genetic evidence of AA-related ancestry in AN speakers might be an artifact of a back-migration from ISEA that contributed ancestry to the H'tin, we removed H'tin from our scaffold tree and repeated our analysis for three-way admixed populations. We found that the formerly H'tin-related ancestry component is now confidently inferred to form a clade with Plang (primarily) or Wa, both of which speak AA languages (Supplementary Table 16). Similarly, when we also removed Plang, it formed a clade with Wa (Supplementary Table 16). We also applied *MixMapper* to two admixed Negrito populations (Jehai and Kensiu) from peninsular Malaysia and found that their Asian ancestry component branches closest to H'tin, in almost exactly the same location as the H'tin-related component from ISEA. Since the Jehai and Kensiu speak AA languages, it is likely that the population contributing their Asian ancestry did as well, and AA-related populations may once have been more widespread in this region. We conclude that our signal indeed reflects gene flow from the mainland into ISEA from an ancestral population that is nested within the radiation of AA-speaking populations, and hence it is likely that this source population itself spoke an AA language.

Discussion

While a major AA contribution to western speakers of AN languages has not been proposed in the genetic literature, results from previous genetic studies are in fact consistent with these findings. A clustering analysis of the Pan-Asia SNP data (25) showed a component of ancestry in populations from (primarily western) ISEA that also appeared in AA speakers on the mainland, and a separate study of the same data also related western ISEA ancestry to mainland

sources (21). However, neither analysis concluded that these signals reflected an AA affinity. Our results are also compatible with published analyses of mtDNA and Y chromosomes, which have provided evidence of a component of ancestry in western but not eastern ISEA that is of Asian origin (20–22). The O-M95 Y-chromosome haplogroup, in particular, is prevalent in western Indonesia (20) and was previously linked to AA-speaking populations (38).

A potential explanation for our detection of AA ancestry in ISEA is that a western stream of AN migrants encountered and mixed with AA speakers in Vietnam or peninsular Malaysia, and it was this mixed population that then settled western Indonesia (Fig. 2). This scenario is consistent with the AN mastery of seafaring technology and would be analogous to the spread of populations of mixed AN and Melanesian ancestry from Near Oceania into Polynesia (13, 15). Since we are unable to determine the date of initial AN–AA admixture, and genetic data from present-day populations do not provide direct information about where historical mixtures occurred, other scenarios are also conceivable; in particular, we cannot formally rule out a wider AA presence in ISEA before the AN expansion or a later diffusion of AA speakers into western ISEA. However, the absence of AA languages in Indonesia, together with our observation of *both* AA and AN ancestry in all surveyed western ISEA populations, suggests that the admixture took place before either group had widely settled the region. We note that in its simplest form, the model of a single early admixture event would imply that populations today should have equal proportions of AN and AA ancestry, which is not the case for our sampled groups. However, these differences could have arisen through a number of straightforward demographic processes, including settlement of different islands by populations with different ancestry proportions, independent fluctuations within populations having heterogeneous ancestry soon after admixture, or continuous or multiple-wave gene flow over a number of generations. Overall, the uniformity of ancestry observed today, with the same components present in all of our sampled groups from western ISEA, points toward a shared mixture event rather than separate events for

each population.

These results show that the AN expansion was not solely a process of cultural diffusion but involved substantial human migrations. The primary movement, reflected today in the universally-present AN ancestry component, involved AN speakers from an ancestral population that is most closely related to present-day aboriginal Taiwanese. In western ISEA, we also find an Asian ancestry component that is unambiguously nested within the variation of present-day AA speakers, which makes it likely that the ancestral population itself spoke an AA language. Other suggestions of AN–AA interaction come from linguistics and archaeology (9), as Bornean AN languages contain probable AA loan words (7), and there is evidence that rice (3, 6, 7, 10) and taro (7) cultivation, as well as domesticated pigs (39), were introduced from the mainland. Interestingly, all languages spoken today in both eastern and western ISEA are part of the AN family, which raises the question of why AN languages were always retained by admixed populations. An important direction for future work is to increase the density of sampling of populations from Southeast Asia, with larger sample sizes and more SNPs, if possible in conjunction with ancient DNA (40), to allow more detailed investigation of the dates and locations of the admixture events we have identified.

Methods

Data set assembly

For our primary analyses, we merged data from the HUGO Pan-Asian SNP Consortium (25) and the CEPH-Human Genome Diversity Panel (HGDP) (29), yielding a set of 1,094 individuals from 56 populations typed at 18,412 overlapping SNPs. We excluded likely duplicate samples, twins, and first-degree relatives from the Pan-Asia data (a total of 79 individuals) as identified in ref. (41). We also removed 27 individuals identified as outliers by projecting each population

onto principal components using EIGENSOFT (42) and deleting samples at least 5 standard deviations away from the population mean on any of the first three PCs.

We also used 10 populations from ref. (27), from a version of the published data set merged with HapMap3 populations but not with Neanderthal and Denisova, for a total of 564,361 SNPs. We restricted to these populations when running *ALDER* and used all of the SNPs. We also merged these samples with our primary data set, leaving 7,668 SNPs, in order to estimate *MixMapper* parameters for Polynesia and Fiji.

In order to test robustness to SNP ascertainment, we repeated our *MixMapper* analyses with a data set formed by merging the Pan-Asia data with HGDP samples typed on the Affymetrix Human Origins array (30), replicating our primary data set on a different collection of 9,032 SNPs. Importantly, the Human Origins SNPs are chosen according to a very different strategy, having been selected based on their presence as heterozygous sites in sequenced genomes from diverse individuals.

Full details for all analyzed populations can be found in Supplementary Table 1.

Admixture inference with *MixMapper*

The *MixMapper* software estimates admixture parameters using allele frequency moment statistics under a tree-based instantaneous admixture model (31). The program works in two phases. First, it constructs an (approximately) unadmixed scaffold tree via neighbor-joining on a subset of populations chosen by the user to have a specified level of geographic coverage with minimal evidence of admixture based on f -statistics (30,37). The selection of populations for the scaffold is guided by running the 3-population test (30,37), which removes clearly admixed populations; by testing the additivity of possible subtrees from among the remaining populations (similar to the 4-population test (30,37)); and finally by comparing the fits of closely related candidate populations when modeled as admixed. After the scaffold is chosen, the software finds

the best-fitting parameters for admixed populations by solving a system of moment equations in terms of the pairwise distance measure f_2 , which is the expected squared allele frequency difference between two populations. Specifically, the distance $f_2(C, X)$ between an admixed population C and each population X on the scaffold tree can be expressed as an algebraic combination of known branch lengths along with four unknown mixture parameters: the locations of the split points of the two ancestral mixing populations from the scaffold tree, the combined terminal branch length, and the mixture fraction α . In this way, the entire tree topology can be determined automatically, even for large numbers of populations. Finally, *MixMapper* uses a non-parametric bootstrap (43) to determine confidence intervals for the parameter estimates, dividing the SNPs into 50 blocks and resampling the blocks at random with replacement for each of 500 replicates. We note that the bootstrap is applied over the entire fitting procedure, including the application of neighbor-joining to build the scaffold, so that uncertainty in the scaffold topology is accounted for in the final confidence intervals.

For our analyses here, we developed new inference algorithms, released in the *MixMapper* 2.0 software, which extend the original *MixMapper* three-way mixture-fitting procedure, whereby one ancestral mixing population is taken to be related to a population already fit by the program as admixed. First, *MixMapper* 2.0 implements a method to determine the best fit among alternative admixture models—namely, fitting a test population C either as two-way admixed or as three-way admixed with one ancestor related to a fixed admixed population A (for our applications, either Manobo or Alorese)—by comparing the norm of the vector of residual errors for all pairwise distances $f_2(C, X)$, where X ranges over the scaffold populations. Importantly, the two models have the same number of degrees of freedom, with four parameters being optimized in each case. Also, the comparison is restricted those populations X on the initial scaffold, i.e., we do not include $f_2(C, A)$ in the vector of residuals for the three-way model. Thus, our procedure is conceptually equivalent to augmenting the scaffold by adding A

(via the standard *MixMapper* admixture model) and then finding the best-fitting placement for *C*. Second, for populations that are better fit as three-way admixed, *MixMapper* 2.0 implements improved estimation of their proportions of ancestry from all three components by re-optimizing this same set of equations but now allowing all of the mixture fractions to vary (as well as the terminal branch lengths for the admixtures, since these depend on the mixture fractions (31)). To prevent overfitting, we fix the branching positions of each ancestry component as determined from the initial fit (independently for each bootstrap replicate).

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Author contributions

All authors contributed to the design of the study and the analysis of data. M.L. and P.L. performed the computational experiments. M.L., P.L., B.B., and D.R. wrote the manuscript with input from all authors.

Competing financial interests

The authors declare no competing financial interests.

References

1. Blust, R. The prehistory of the Austronesian-speaking peoples: a view from language. *J. World Prehist.* **9**, 453–510 (1995).
2. Gray, R., Drummond, A. & Greenhill, S. Language phylogenies reveal expansion pulses and pauses in Pacific settlement. *Science* **323**, 479–483 (2009).
3. Bellwood, P. *Prehistory of the Indo-Malaysian Archipelago* (Univ. Hawai'i Press, Honolulu, 1997).
4. Diamond, J. & Bellwood, P. Farmers and their languages: the first expansions. *Science* **300**, 597–603 (2003).
5. Bellwood, P. *First farmers: the origins of agricultural societies* (Blackwell, Oxford, 2005).
6. Donohue, M. & Denham, T. Farming and language in Island Southeast Asia: Reframing Austronesian history. *Curr. Anthropol.* **51**, 223–256 (2010).
7. Blench, R. Was there an Austroasiatic presence in Island Southeast Asia prior to the Austronesian expansion? *Bull. Indo-Pacific Prehist. Assoc.* **30**, 133–144 (2011).
8. Barker, G. & Richards, M. B. Foraging–farming transitions in Island Southeast Asia. *J. Arch. Method and Theory* **20**, 256–280 (2013).
9. Anderson, A. Crossing the Luzon Strait: Archaeological chronology in the Batanes Islands, Philippines and the regional sequence of Neolithic dispersal. *J. Austronesian Stud.* **1**, 25–45 (2005).

10. Bellwood, P., Chambers, G., Ross, M. & Hung, H. Are ‘cultures’ inherited? Multidisciplinary perspectives on the origins and migrations of Austronesian-speaking peoples prior to 1000 BC. In Roberts, B. & Vander Linden, M. (eds.) *Investigating Archaeological Cultures*, 321–354 (Springer, New York, 2011).
11. Melton, T. *et al.* Polynesian genetic affinities with Southeast Asian populations as identified by mtDNA analysis. *Am. J. Hum. Genet.* **57**, 403–414 (1995).
12. Sykes, B., Leiboff, A., Low-Beer, J., Tetzner, S. & Richards, M. The origins of the Polynesians: an interpretation from mitochondrial lineage analysis. *Am. J. Hum. Genet.* **57**, 1463–1475 (1995).
13. Kayser, M. *et al.* Melanesian origin of Polynesian Y chromosomes. *Curr. Biol.* **10**, 1237–1246 (2000).
14. Trejaut, J. *et al.* Traces of archaic mitochondrial lineages persist in Austronesian-speaking Formosan populations. *PLoS Biol.* **3**, e247 (2005).
15. Kayser, M. *et al.* The impact of the Austronesian expansion: evidence from mtDNA and Y chromosome diversity in the Admiralty Islands of Melanesia. *Mol. Biol. Evol.* **25**, 1362–1374 (2008).
16. Su, B. *et al.* Polynesian origins: Insights from the Y chromosome. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 8225–8228 (2000).
17. Oppenheimer, S. & Richards, M. Polynesian origins: slow boat to Melanesia? *Nature* **410**, 166–167 (2001).
18. Soares, P. *et al.* Ancient voyaging and Polynesian origins. *Am. J. of Hum. Genet.* **88**, 239–247 (2011).

19. Hill, C. *et al.* A mitochondrial stratigraphy for island Southeast Asia. *Am. J. Hum. Genet.* **80**, 29–43 (2007).
20. Karafet, T. *et al.* Major east–west division underlies Y chromosome stratification across Indonesia. *Mol. Biol. Evol.* **27**, 1833–1844 (2010).
21. Jinam, T. *et al.* Evolutionary history of continental Southeast Asians: “Early train” hypothesis based on genetic analysis of mitochondrial and autosomal DNA data. *Mol. Biol. Evol.* **29**, 3513–3527 (2012).
22. Tumonggor, M. *et al.* The Indonesian archipelago: an ancient genetic highway linking Asia and the Pacific. *J. Hum. Genet.* **58**, 165–173 (2013).
23. Friedlaender, J. *et al.* The genetic structure of Pacific Islanders. *PLoS Genet.* **4**, e19 (2008).
24. Xu, S. *et al.* Genetic dating indicates that the Asian–Papuan admixture through eastern Indonesia corresponds to the Austronesian expansion. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 4574–4579 (2012).
25. HUGO Pan-Asian SNP Consortium. Mapping human genetic diversity in Asia. *Science* **326**, 1541–1545 (2009).
26. Cox, M., Karafet, T., Lansing, J., Sudoyo, H. & Hammer, M. Autosomal and X-linked single nucleotide polymorphisms reveal a steep Asian–Melanesian ancestry cline in eastern Indonesia and a sex bias in admixture rates. *Proc. R. Soc. London Ser. B* **277**, 1589–1596 (2010).
27. Reich, D. *et al.* Denisova admixture and the first modern human dispersals into Southeast Asia and Oceania. *Am. J. Hum. Genet.* **89**, 516–528 (2011).

28. Pierron, D. *et al.* Genome-wide evidence of Austronesian–Bantu admixture and cultural reversion in a hunter-gatherer group of Madagascar. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 936–941 (2014).
29. Li, J. *et al.* Worldwide human relationships inferred from genome-wide patterns of variation. *Science* **319**, 1100–1104 (2008).
30. Patterson, N. *et al.* Ancient admixture in human history. *Genetics* **192**, 1065–1093 (2012).
31. Lipson, M. *et al.* Efficient moment-based inference of admixture parameters and sources of gene flow. *Mol. Biol. Evol.* **30**, 1788–1802 (2013).
32. Pickrell, J. & Pritchard, J. Inference of population splits and mixtures from genome-wide allele frequency data. *PLoS Genet.* **8**, e1002967 (2012).
33. Reich, D. *et al.* Reconstructing Native American population history. *Nature* **488**, 370–374 (2012).
34. Moorjani, P. *et al.* Genetic evidence for recent population mixture in India. *Am. J. Hum. Genet.* **93**, 422–438 (2013).
35. Loh, P.-R. *et al.* Inferring admixture histories of human populations using linkage disequilibrium. *Genetics* **193**, 1233–1254 (2013).
36. Fenner, J. Cross-cultural estimation of the human generation interval for use in genetics-based population divergence studies. *Am. J. Phys. Anthropol.* **128**, 415–423 (2005).
37. Reich, D., Thangaraj, K., Patterson, N., Price, A. & Singh, L. Reconstructing Indian population history. *Nature* **461**, 489–494 (2009).

38. Kumar, V. *et al.* Y-chromosome evidence suggests a common paternal heritage of Austro-Asiatic populations. *BMC Evol. Biol.* **7**, 47 (2007).
39. Larson, G. *et al.* Phylogeny and ancient DNA of *Sus* provides insights into neolithic expansion in Island Southeast Asia and Oceania. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 4834–4839 (2007).
40. Ko, A. M.-S. *et al.* Early Austronesians: into and out of Taiwan. *Am. J. Hum. Genet.* **94**, 426–436 (2014).
41. Yang, X. & Xu, S. Identification of close relatives in the HUGO Pan-Asian SNP Database. *PloS one* **6**, e29502 (2011).
42. Patterson, N., Price, A. & Reich, D. Population structure and eigenanalysis. *PLoS Genet.* **2**, e190 (2006).
43. Efron, B. & Tibshirani, R. Bootstrap methods for standard errors, confidence intervals, and other measures of statistical accuracy. *Stat. Sci.* **1**, 54–75 (1986).
44. The International HapMap Consortium. Integrating common and rare genetic variation in diverse human populations. *Nature* **467**, 52–58 (2010).

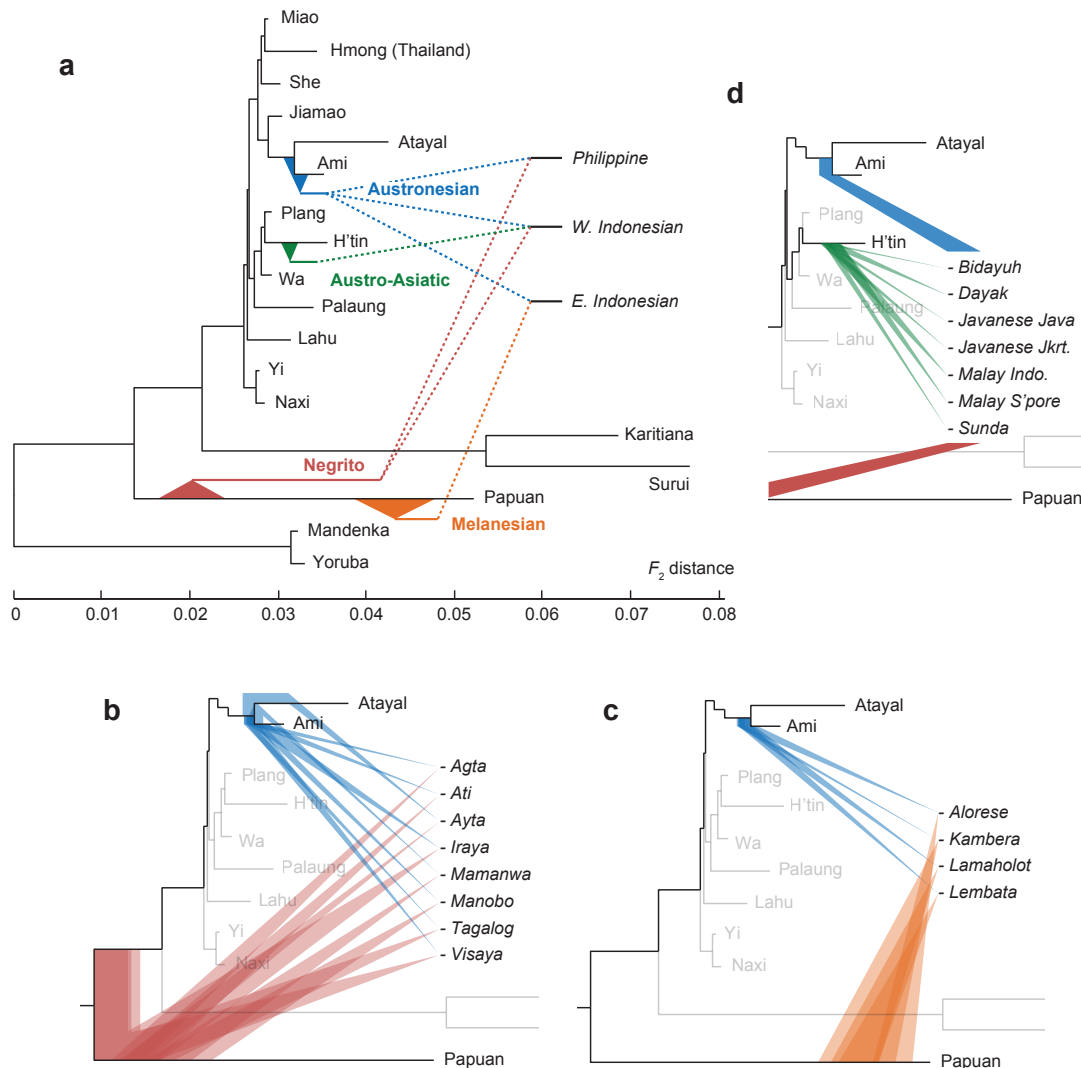


Figure 1. Inferred sources of ancestry for selected admixed Austronesian-speaking populations. Shaded ranges represent 95% bootstrap confidence intervals for branching positions; see Supplementary Tables 4 and 5 for complete mixing branch distributions. The topology of the scaffold tree is shown using the full data set (slight variations are possible across bootstrap replicates). (A) Overview of the three best-fitting admixture models. (B)–(D) Detailed results for highest-confidence models of populations from (B) the Philippines, (C) eastern Indonesia, and (D) western ISEA. In (D), the Austronesian and Negrito branch positions are fixed in *MixMapper* to equal those for Manobo. Batak Toba are omitted for display purposes, as 8% of replicates place their third ancestry component on a non-adjacent branch in the scaffold (Supplementary Table 5). Three other populations (Manggarai Ngada, Manggarai Rampasasa, and Toraja) fall into an additional category of three-way admixed eastern Indonesians, while Oceanians (Fiji and Polynesia) are inferred to have similar ancestry to the populations in (C), but their confidence intervals are not directly comparable because they have fewer SNPs available (see Fig. 2 and Supplementary Tables 4 and 5).

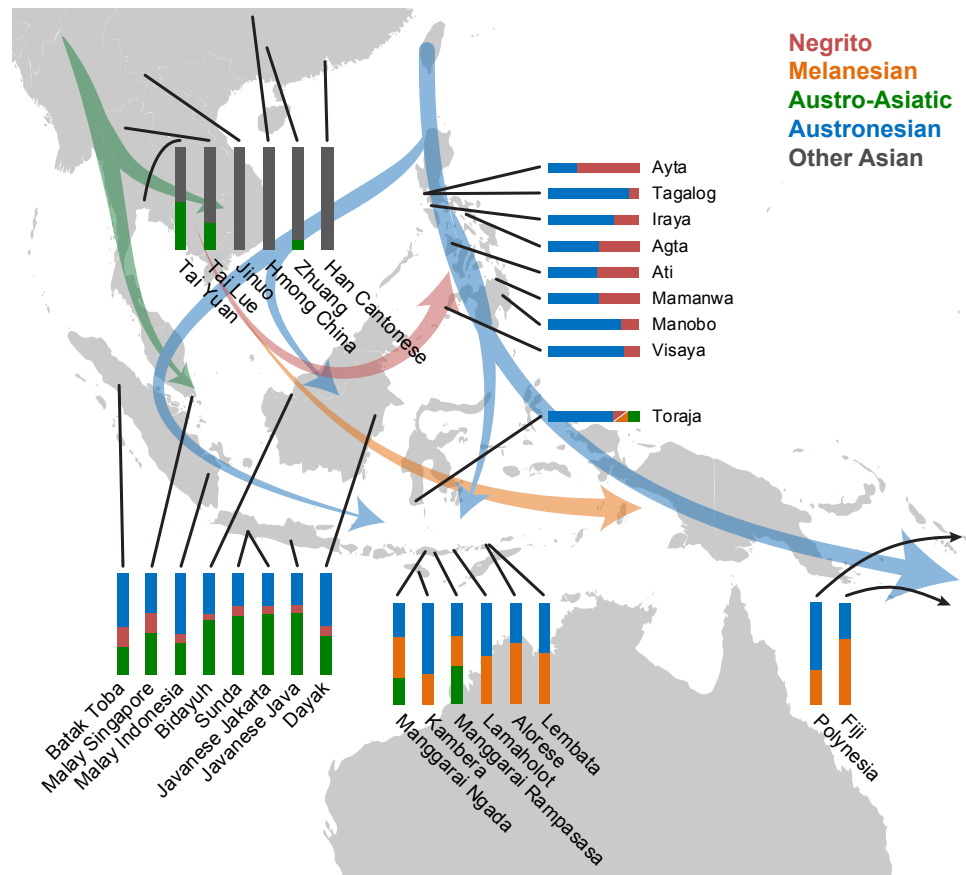
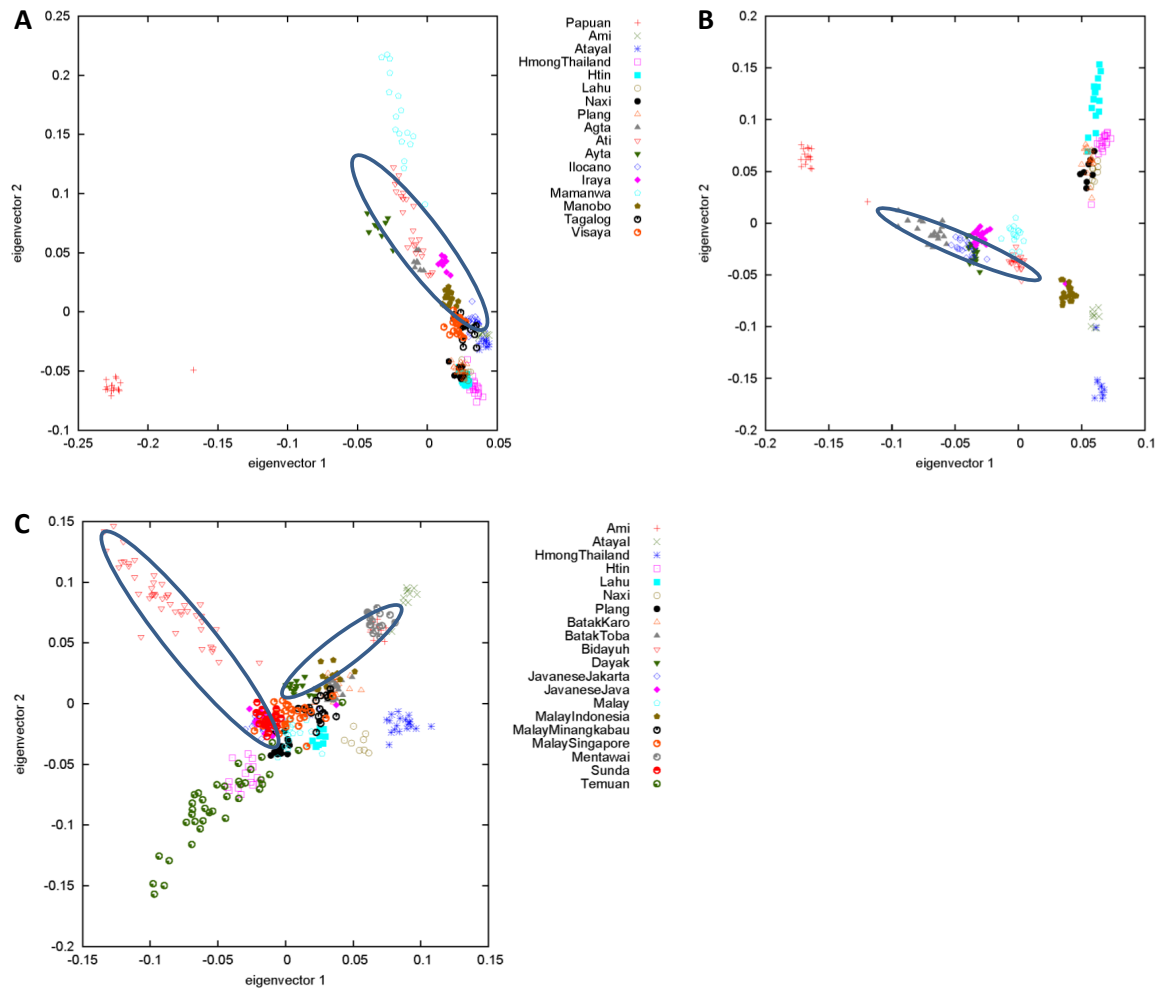


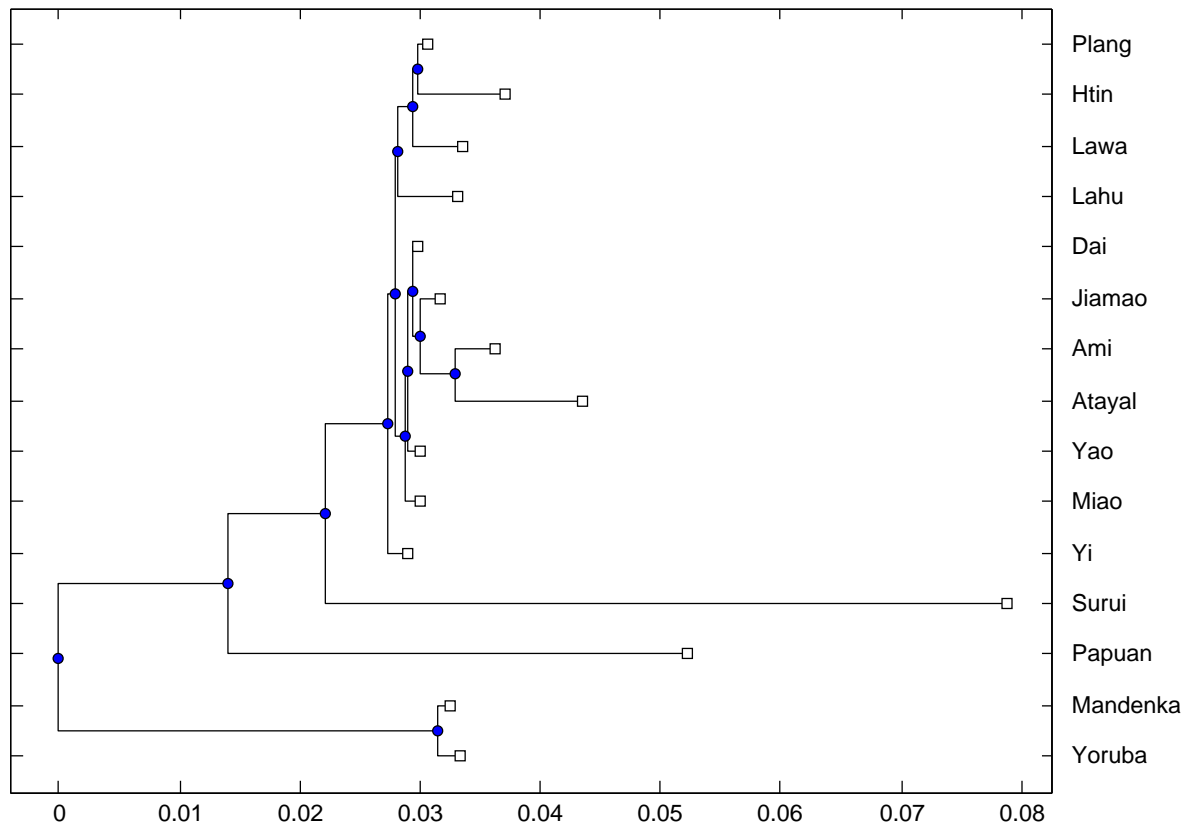
Figure 2. Locations and best-fit mixture proportions (see Methods) for Austronesian-speaking and other populations, with possible directions of human migrations supported by our analyses. For Toraja, we could not distinguish between Negrito and Melanesian ancestry and show this component as red/orange.

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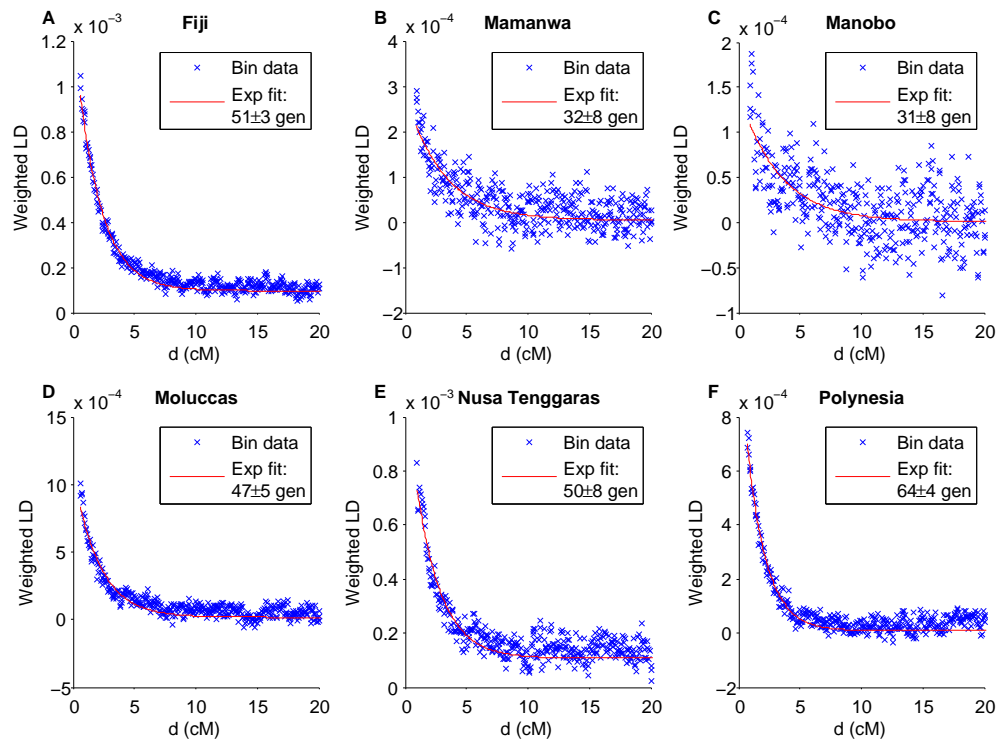
Mark Lipson, Po-Ru Loh, Nick Patterson, Priya Moorjani,
Ying-Chin Ko, Mark Stoneking, Bonnie Berger, and David Reich



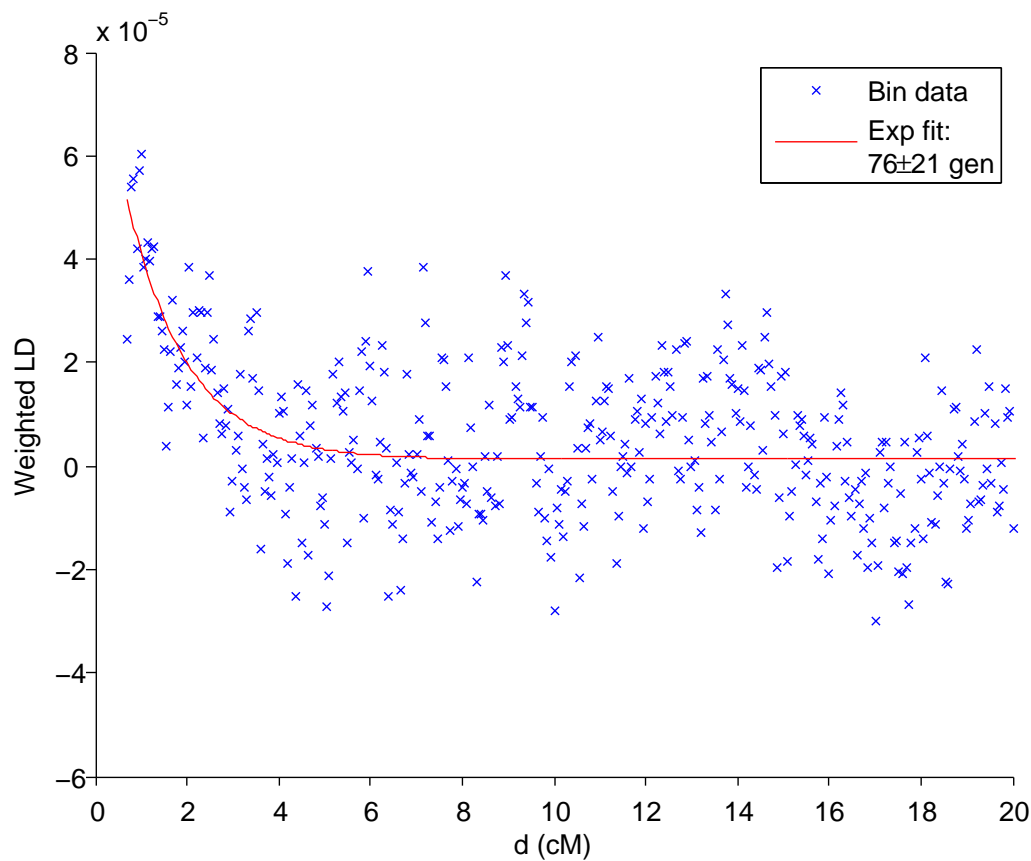
Supplementary Figure 1. PCA plots generated with EIGENSOFT (42) for AN-speaking groups from (A) the Philippines, (B) eastern Indonesia, and (C) western ISEA, along with reference populations. The circled groupings indicate subsets of populations consistent with simple histories according to our f_4 -based test: (A) Agta, Ati, Ayta, Ilocano, Iraya, and Manobo (one wave of admixture), (B) Alorese, Kambera, Lamaholot, and Lembata (one wave), and (C) Bidayuh, Dayak, Mentawai, Javanese Jakarta, Javanese Java, and Sunda (two waves).



Supplementary Figure 2. Alternative 15-population scaffold tree. See Supplementary Tables 8 and 9 for full *MixMapper* results from fitting admixed Austronesian-speaking populations using this scaffold. Distances are in F_2 units.



Supplementary Figure 3. Weighted LD curves and estimated dates of admixture for (A) Fiji, (B) Mamanwa, (C) Manobo, (D) Moluccas, (E) Nusa Tenggara, and (F) Polynesia, obtained using *ALDER* (35) with Papuan and Taiwanese reference populations. Admixture dates are inferred as time constants of the exponential decay of weighted covariance with genetic distance. LD analysis requires a higher SNP density than is available with our full data set, so these inferences are restricted to samples from ref. (27). We note that our dates are much more recent than those reported in ref. (24); we hypothesize that the initial admixtures were followed by more recent mixing between groups with different proportions of Taiwan-related ancestry, in which case the date from *ALDER* is an intermediate one over the entire process. This would be consistent with the fact that the curves appear to have some deviations from a pure exponential decay shape.



Supplementary Figure 4. Weighted LD curve and estimated date of admixture for western ISEA, obtained using *ALDER* (35) with Papuan and CHB (HapMap Chinese from Beijing (44)) reference populations. The admixture date is inferred as the time constant of the exponential decay of weighted covariance with genetic distance. LD analysis requires a higher SNP density than is available with our full data set, so these inferences are restricted to samples from ref. (27). In order to enhance the signal-to-noise ratio, we pooled samples from four populations, two each from Borneo (Bidayuh and Dayak) and Sumatra (Besemah and Semende), into a single test set, under the assumption that all four have similar admixture histories.

Supplementary Table 1. Summary of populations used in this study

Population	Country	Data set	Pan-Asia ID	# samples	Model status
Ami	Taiwan	Pan-Asia	AX-AM	10	Scaffold
Atayal	Taiwan	Pan-Asia	AX-AT	10	Scaffold
Miao	China	HGDP		10	Scaffold
She	China	HGDP		10	Scaffold
Jiamao	China	Pan-Asia	CN-JI	31	Scaffold
Lahu	China	HGDP		8	Scaffold
Wa	China	Pan-Asia	CN-WA	50	Scaffold
Yi	China	HGDP		10	Scaffold
Naxi	China	HGDP		8	Scaffold
Hmong	Thailand	Pan-Asia	TN-HM	20	Scaffold
Plang	Thailand	Pan-Asia	TH-PP	18	Scaffold
H'tin	Thailand	Pan-Asia	TH-TN	15	Scaffold
Palaung	Thailand	Pan-Asia	TH-PL	18	Scaffold
Karitiana	Brazil	HGDP		14	Scaffold
Surui	Brazil	HGDP		8	Scaffold
Papuan	Papua New Guinea	HGDP		17	Scaffold
Mandenka	Senegal	HGDP		22	Scaffold
Yoruba	Nigeria	HGDP		21	Scaffold
Aboriginal Taiwanese	Taiwan	Reich et al. (2011)		10	ALDER reference
CHB	China	HapMap Phase 3 (44)		88	ALDER reference
Papuan ¹	Papua New Guinea	Reich et al. (2011)		24	ALDER reference
Aqta	Philippines	Pan-Asia	PI-AG	8	Three-way admixed
Ati	Philippines	Pan-Asia	PI-AT	23	Three-way admixed
Ayta	Philippines	Pan-Asia	PI-AE	8	Two-way admixed
Iraya	Philippines	Pan-Asia	PI-IR	9	Two-way admixed
Mamanwa	Philippines	Pan-Asia	PI-MW	17	Two-way admixed
Mamanwa ¹	Philippines	Reich et al. (2011)		11	Two-way admixed
Manobo	Philippines	Pan-Asia	PI-MA	18	Two-way admixed
Manobo ¹	Philippines	Reich et al. (2011)		16	Two-way admixed
Tagalog	Philippines	Pan-Asia	PI-UN	19	Two-way admixed
Visaya	Philippines	Pan-Asia	PI-UI	20	Three-way admixed
Alorese	Indonesia	Pan-Asia	ID-AL	19	Two-way admixed
Kambera	Indonesia	Pan-Asia	ID-SB	20	Two-way admixed
Lamaholot	Indonesia	Pan-Asia	ID-LA	20	Three-way admixed
Lembata	Indonesia	Pan-Asia	ID-LE	19	Three-way admixed
Manggarai Ngada	Indonesia	Pan-Asia	ID-SO	19	Three-way admixed
Manggarai Rampasasa	Indonesia	Pan-Asia	ID-RA	16	Three-way admixed
Fiji	Fiji	Reich et al. (2011)		25	Two-way admixed
Polynesia	Multiple ²	Reich et al. (2011)		19	Two-way admixed
Toraja	Indonesia	Pan-Asia	ID-TR	20	Three-way admixed
Moluccas	Indonesia	Reich et al. (2011)		10	Two-way admixed
Nusa Tenggara	Indonesia	Reich et al. (2011)		10	Two-way admixed
Batak Toba	Indonesia	Pan-Asia	ID-TB	20	Three-way admixed
Bidayuh	Malaysia	Pan-Asia	MY-BD	47	Three-way admixed
Bidayuh ¹	Malaysia	Reich et al. (2011)		10	Three-way admixed
Dayak	Indonesia	Pan-Asia	ID-DY	12	Three-way admixed
Dayak ¹	Indonesia	Reich et al. (2011)		16	Three-way admixed
Javanese Jakarta	Indonesia	Pan-Asia	ID-JA	34	Three-way admixed
Javanese Java	Indonesia	Pan-Asia	ID-JV	19	Three-way admixed
Malay Indonesia	Indonesia	Pan-Asia	ID-ML	12	Three-way admixed
Malay Singapore	Singapore	Pan-Asia	SG-MY	28	Three-way admixed
Sunda	Indonesia	Pan-Asia	ID-SU	25	Three-way admixed
Besemah	Indonesia	Reich et al. (2011)		8	Three-way admixed
Semende	Indonesia	Reich et al. (2011)		9	Three-way admixed
Batak Karo	Indonesia	Pan-Asia	ID-KR	17	Uncertain admixed
Malay	Malaysia	Pan-Asia	MY-KN	18	Uncertain admixed
Malay Minangkabau	Malaysia	Pan-Asia	MY-MN	19	Uncertain admixed
Mentawai	Indonesia	Pan-Asia	ID-MT	15	Uncertain admixed
Ilocano	Philippines	Pan-Asia	PI-UB	20	Uncertain admixed
Temuan	Malaysia	Pan-Asia	MY-TM	37	Uncertain admixed
Melanesian	Papua New Guinea	HGDP		10	Uncertain admixed
Jehai	Malaysia	Pan-Asia	MY-JH	42	Two-way admixed
Kensiu	Malaysia	Pan-Asia	MY-KS	25	Two-way admixed
Zhuang	China	Pan-Asia	CN-CC	24	Other mainland
Jinuo	China	Pan-Asia	CN-JN	29	Other mainland
Han Cantonese	China	Pan-Asia	CN-GA	28	Other mainland
Hmong	China	Pan-Asia	CN-HM	20	Other mainland
Tai Lue	Thailand	Pan-Asia	TH-TL	18	Other mainland
Tai Yuan	Thailand	Pan-Asia	TH-TU	20	Other mainland

[Caption on next page.]

Caption for Supplementary Table 1:

Summary of population samples used in this study. The first group of populations are references used in the 18-population scaffold tree and for admixture date estimation, the second group are Austronesian-speaking populations fit as admixtures, and the third group are other populations used for comparison.

¹Samples used for admixture date inference with *ALDER* were taken from Reich et al. (2011) rather than from Pan-Asia or HGDP for the main *MixMapper* analysis.

²The Polynesian samples are from the Cook Islands (2), Futuna (4), Niue (1), Samoa (5), Tokelau (2), Tonga (2), and Tuvalu (3).

Supplementary Table 2. Populations with negative f_3 statistics

Test population C	Reference population A	Reference population B	$f_3(C; A, B)$	Std error	Z-score
Alorese	Tagalog	Papuan	-0.0106	0.00023	-45.36
Batak Karo	Mentawai	Kalash	-0.00179	0.00025	-7.21
Batak Toba	Mentawai	Tuscan	-0.00238	0.00024	-9.96
Han Cantonese	Korean	Jiamao	-0.00079	0.00007	-12.04
Hmong China	Hmong Thailand	Mbuti Pygmy	-0.00132	0.0002	-6.55
Ilocano	Ami	Bengali	-0.00098	0.00024	-4.14
Javanese Jakarta	Ilocano	Jehai	-0.00113	0.00015	-7.43
Javanese Java	Ami	Jehai	-0.00133	0.00017	-7.69
Kambera	Tagalog	Papuan	-0.00719	0.00025	-29.26
Lamaholot	Toraja	Papuan	-0.0091	0.00022	-41.2
Lembata	Toraja	Papuan	-0.00961	0.00022	-43.26
Malay	Zhuang	GIH	-0.00322	0.00013	-24.5
Malay Indonesia	Ami	Bengali	-0.00201	0.00028	-7.19
Malay Minangkabau	Ami	Hindi Haryana	-0.00262	0.00026	-9.97
Malay Singapore	Hindi Haryana	Jiamao	-0.00209	0.00011	-18.66
Manggarai Ngada	Tagalog	Papuan	-0.00883	0.00025	-35.49
Manggarai Rampasasa	Ilocano	Papuan	-0.00682	0.00029	-23.88
Manobo	Ami	Papuan	-0.0006	0.00035	-1.7
Miao	Hmong Thailand	Colombian	-0.0004	0.00028	-1.41
Plang	Mlabri	Han-NChina	-0.00021	0.00027	-0.78
Sunda	Ilocano	Jehai	-0.00113	0.00014	-8.15
Tagalog	Ami	Hindi Rajasthan	-0.00214	0.00019	-11.11
Tai Yuan	Htin	CHB	-0.00082	0.00008	-9.76
Toraja	Ilocano	Papuan	-0.00213	0.00026	-8.18
Visaya	Ami	Hindi Rajasthan	-0.00298	0.0002	-14.79
Wa	Mlabri	Naxi	-0.00005	0.00028	-0.16
Yi	Mlabri	Naxi	-0.00006	0.00034	-0.18
Zhuang	Jiamao	Lahu	-0.0002	0.0001	-1.91

Asian populations from Supplementary Table 1 having at least one negative f_3 value. For each test population C , we show the two reference populations A and B in the data set giving the lowest Z-score for $f_3(C; A, B)$. We note that all populations on this list that are used in the scaffold have $Z > -2$, which indicates a non-significant result (especially given the presence of many hypotheses). While a significantly negative f_3 value demonstrates that the test population must be admixed, a lack of a negative value does not prove a lack of admixture.

Supplementary Table 3. Populations with no negative f_3 statistics

Agta
Ami
Atayal
Ati
Ayta
Bidayuh
Dayak
Hmong Thailand
Htin
Iraya
Jehai
Jiamao
Jinuo
Kensiu
Lahu
Mamanwa
Mentawai
Mlabri
Naxi
Paluang
She
Tai Lue
Temuan

Asian populations from Supplementary Table 1 having no negative f_3 value for any pair of reference populations in the data set. While a significantly negative f_3 value demonstrates that the test population must be admixed, a lack of a negative value does not prove a lack of admixture.

Supplementary Table 4. Inferred mixture parameters for two-way admixed populations

Philippine admixed population	Mixing branch 1 bootstrap distribution		Mixing branch 2 bootstrap distribution		Branch 1 ancestry (Austronesian)
Agta	(Ami,Atayal)	44%	Papuan	100%	51–62%
	Ami	56%			
Ati	(Ami,Atayal)	15%	Papuan	100%	50–59%
	Ami	85%			
Ayta	(Ami,Atayal)	20%	Papuan	100%	25–38%
	Ami	7%			
	Atayal	73%			
Iraya	(Ami,Atayal)	28%	Papuan	76%	61–80%
	Ami	72%	Papuan opp. African	20%	
Mamanwa	(Ami,Atayal)	25%	Papuan	100%	51–61%
	Ami	62%			
	Atayal	13%			
Manobo	(Ami,Atayal)	11%	Papuan	100%	78–83%
	Ami	89%			
Tagalog	(Ami,Atayal)	99%	Papuan	71%	83–92%
			Papuan opp. African	28%	
Visaya	(Ami,Atayal)	88%	Papuan	85%	74–85%
	Ami	11%	Papuan opp. African	15%	
E. Indonesian / Oceanian admixed population	Mixing branch 1 bootstrap distribution		Mixing branch 2 bootstrap distribution		Branch 1 ancestry (Austronesian)
Alorese	(Ami,Atayal)	77%	Papuan	100%	37–44%
	Ami	17%			
	Atayal	6%			
Fiji	(Ami,Atayal)	19%	Papuan	100%	30–41%
	Ami	64%			
	Atayal	17%			
Kambera	(Ami,Atayal)	100%	Papuan	100%	67–73%
Lamaholot	(Ami,Atayal)	93%	Papuan	100%	50–56%
	Ami	6%			
Lembata	(Ami,Atayal)	94%	Papuan	100%	47–53%
Polynesia	(Ami,Atayal)	20%	Papuan	100%	61–72%
	Ami	54%			
	Atayal	26%			

Sources of ancestry and mixture proportions (95% confidence intervals) from *MixMapper* for two-way admixed populations. “Papuan opp. African” refers to the common ancestral branch of all populations in the scaffold other than Papuan and Africans, while (Ami, Atayal) designates the common ancestral branch of Ami and Atayal (see Fig. 1). Branch topologies are shown that occur for at least 5% of 500 bootstrap replicates.

Supplementary Table 5. Inferred mixture parameters for three-way admixed populations

E. Indonesian / Oceanian admixed population	Percent bootstrap reps with Branch 3 = H'tin	Branch 3 ancestry (Austro-Asiatic)	Branch 1 ancestry (Austronesian)
Manggarai Ngada	100%	24–29%	31–37%
Manggarai Rampasasa	100%	34–41%	29–37%
Toraja	100%	10–17%	68–75%
W. Indonesian admixed population	Percent bootstrap reps with Branch 3 = H'tin	Branch 3 ancestry (Austro-Asiatic)	Branch 1 ancestry (Austronesian)
Batak Toba	92%	22–32%	50–57%
Bidayuh	100%	50–57%	37–44%
Dayak	100%	35–42%	48–56%
Javanese Jakarta	100%	57–63%	29–35%
Javanese Java	100%	57–64%	28–34%
Malay Indonesia	100%	26–34%	56–64%
Malay Singapore	100%	38–45%	37–43%
Sunda	100%	54–61%	30–36%

Mixture parameters from *MixMapper* for three-way admixed populations. Mixture proportions shown are 95% confidence intervals for re-optimized values (see Methods), using the bootstrap replicates (percentages given, out of 500) assigning the third ancestry component to the H'tin branch.

Supplementary Table 6. Inferred mixture parameters for two-way admixed populations with alternative SNP ascertainment

Philippine admixed population	Mixing branch 1 bootstrap distribution	Mixing branch 2 bootstrap distribution	Branch 1 ancestry (Austronesian)
Agta	(Ami,Atayal) 51% Ami 38% Atayal 11%	Papuan 62%	51–66%
Ati	(Ami,Atayal) 93%	Papuan 100%	53–68%
Ayta	(Ami,Atayal) 31% Ami 17% Atayal 48%	Papuan 89%	23–45%
Iraya	(Ami,Atayal) 29% Ami 59% Atayal 12%	Papuan 35% Papuan opp. African 6%	60–86%
Mamanwa	(Ami,Atayal) 41% Ami 42% Atayal 17%	Papuan 100%	49–66%
Manobo	(Ami,Atayal) 33% Ami 66%	Papuan 100%	77–87%
Tagalog	(Ami,Atayal) 98%	Papuan 78%	85–93%
Visaya	(Ami,Atayal) 87% Ami 5% Atayal 8%	Papuan 99%	82–91%
E. Indonesian / Oceanian admixed population	Mixing branch 1 bootstrap distribution	Mixing branch 2 bootstrap distribution	Branch 1 ancestry (Austronesian)
Alorese	(Ami,Atayal) 72% Atayal 20%	Papuan 100%	38–47%
Kambera	(Ami,Atayal) 95%	Papuan 100%	65–75%
Lamaholot	(Ami,Atayal) 93%	Papuan 100%	51–62%
Lembata	(Ami,Atayal) 55% Ami 15% Atayal 30%	Papuan 100%	48–57%

Sources of ancestry and mixture proportions (95% confidence intervals) from *MixMapper* for two-way admixed populations, using SNPs selected by merging the Pan-Asia data with HGDP samples typed on the Affymetrix Human Origins array (30). “Papuan opp. African” refers to the common ancestral branch of all populations in the scaffold other than Papuan and Africans, while (Ami, Atayal) designates the common ancestral branch of Ami and Atayal (see Fig. 1). Branch topologies are shown that occur for at least 5% of 500 bootstrap replicates. The results are very similar to those obtained with the original scaffold (see Supplementary Table 4).

Supplementary Table 7. Inferred mixture parameters for three-way admixed populations with alternative SNP ascertainment

E. Indonesian / Oceanian admixed population	Percent bootstrap reps with Branch 3 = H'tin	Branch 3 ancestry (Austro-Asiatic)	Branch 1 ancestry (Austronesian)
Manggarai Ngada	66%	20–31%	29–42%
Manggarai Rampasasa	27%	29–38%	33–44%
Toraja	85%	6–14%	70–79%
W. Indonesian admixed population	Percent bootstrap reps with Branch 3 = H'tin	Branch 3 ancestry (Austro-Asiatic)	Branch 1 ancestry (Austronesian)
Batak Toba	28%	19–35%	49–60%
Bidayuh	99%	42–58%	36–50%
Dayak	98%	27–44%	46–59%
Javanese Jakarta	100%	49–64%	28–40%
Javanese Java	100%	52–70%	24–38%
Malay Indonesia	76%	18–33%	58–73%
Malay Singapore	74%	29–49%	35–51%
Sunda	100%	50–65%	27–41%

Mixture parameters from *MixMapper* for three-way admixed populations, using SNPs selected by merging the Pan-Asia data with HGDP samples typed on the Affymetrix Human Origins array (30). Mixture proportions shown are 95% confidence intervals for re-optimized values (see Methods), using the bootstrap replicates (percentages given, out of 500) assigning the third ancestry component to the H'tin branch. The results are very similar to those obtained with the original scaffold (see Supplementary Table 5), with slightly lower but still substantial bootstrap support for the H'tin-related ancestry component.

Supplementary Table 8. Inferred mixture parameters for two-way admixed populations on a 15-population alternative scaffold

Philippine admixed population	Mixing branch 1 bootstrap distribution		Mixing branch 2 bootstrap distribution		Branch 1 ancestry (Austronesian)
Agta	(Ami,Atayal)	44%	Papuan	100%	50–60%
	Ami	56%			
Ati	(Ami,Atayal)	12%	Papuan	100%	49–58%
	Ami	88%			
Ayta	(Ami,Atayal)	21%	Papuan	100%	24–37%
	Ami	7%			
	Atayal	72%			
Iraya	(Ami,Atayal)	16%	Papuan	39%	56–78%
	Ami	84%	Papuan opp. African	60%	
Mamanwa	(Ami,Atayal)	40%	Papuan	100%	51–61%
	Ami	53%			
	Atayal	7%			
Manobo	(Ami,Atayal)	9%	Papuan	100%	78–83%
	Ami	91%			
Tagalog	(Ami,Atayal)	100%	Papuan	64%	83–92%
			Papuan opp. African	34%	
Visaya	(Ami,Atayal)	82%	Papuan	78%	72–85%
	Ami	18%	Papuan opp. African	22%	
E. Indonesian / Oceanian admixed population	Mixing branch 1 bootstrap distribution		Mixing branch 2 bootstrap distribution		Branch 1 ancestry (Austronesian)
Alorese	(Ami,Atayal)	84%	Papuan	100%	37–43%
	Ami	14%			
Fiji	(Ami,Atayal)	16%	Papuan	100%	30–40%
	Ami	66%			
	Atayal	18%			
Kambera	(Ami,Atayal)	100%	Papuan	100%	68–72%
Lamaholot	(Ami,Atayal)	96%	Papuan	100%	49–56%
Lembata	(Ami,Atayal)	98%	Papuan	100%	47–53%
Polynesia	(Ami,Atayal)	23%	Papuan	100%	61–72%
	Ami	52%			
	Atayal	25%			

Sources of ancestry and mixture proportions (95% confidence intervals) from *MixMapper* for two-way admixed populations using a 15-population alternative scaffold tree. The results are very similar to those obtained with the original scaffold (see Supplementary Table 4). “Papuan opp. African” refers to the common ancestral branch of all populations in the scaffold other than Papuan and Africans, while (Ami, Atayal) designates the common ancestral branch of Ami and Atayal (see Fig. 1). Branch topologies are shown that occur for at least 5% of 500 bootstrap replicates.

Supplementary Table 9. Inferred mixture parameters for three-way admixed populations on a 15-population alternative scaffold

E. Indonesian / Oceanian admixed population	Percent bootstrap reps with Branch 3 = H'tin	Branch 3 ancestry (Austro-Asiatic)	Branch 1 ancestry (Austronesian)
Manggarai Ngada	83%	24–30%	30–36%
Manggarai Rampasasa	81%	35–43%	28–36%
Toraja	90%	7–17%	68–77%
W. Indonesian admixed population	Percent bootstrap reps with Branch 3 = H'tin	Branch 3 ancestry (Austro-Asiatic)	Branch 1 ancestry (Austronesian)
Batak Toba	52%	23–33%	49–57%
Bidayuh	100%	52–62%	33–43%
Dayak	100%	35–44%	46–56%
Javanese Jakarta	100%	59–66%	27–33%
Javanese Java	100%	60–69%	25–33%
Malay Indonesia	87%	26–36%	54–65%
Malay Singapore	68%	40–47%	35–42%
Sunda	100%	58–65%	26–33%

Mixture parameters from *MixMapper* for three-way admixed populations using a 15-population alternative scaffold tree. The results are very similar to those obtained with the original scaffold (see Supplementary Table 5), with slightly lower but still substantial bootstrap support for the H'tin-related ancestry component. Mixture proportions shown are 95% confidence intervals for re-optimized values (see Methods), using the bootstrap replicates (percentages given, out of 500) assigning the third ancestry component to the H'tin branch.

Supplementary Table 10. Consistency of mixture parameters for two-way admixed populations on 17-population alternative scaffolds

Philippine admixed population	Taiwan bootstrap support	Papuan bootstrap support	Taiwan ancestry fraction
Agta	100 ± 0%	99 ± 0%	56 ± 1%
Ati	100 ± 0%	100 ± 0%	55 ± 0%
Ayta	99 ± 1%	100 ± 0%	32 ± 1%
Iraya	100 ± 0%	79 ± 8%	73 ± 2%
Mamanwa	100 ± 0%	100 ± 0%	56 ± 0%
Manobo	100 ± 0%	100 ± 0%	81 ± 0%
Tagalog	100 ± 0%	69 ± 11%	89 ± 0%
Visaya	100 ± 0%	83 ± 6%	83 ± 0%
E. Indonesian / Oceanian admixed population	Taiwan bootstrap support	Papuan bootstrap support	Taiwan ancestry fraction
Alorese	100 ± 0%	100 ± 0%	40 ± 0%
Fiji	100 ± 1%	100 ± 0%	36 ± 0%
Kambera	100 ± 0%	100 ± 0%	70 ± 1%
Lamaholot	100 ± 0%	100 ± 0%	53 ± 1%
Lembata	100 ± 0%	100 ± 0%	50 ± 1%
Polynesia	100 ± 0%	100 ± 0%	66 ± 0%

Sources of ancestry and mixture proportions (95% confidence intervals) from *MixMapper* for two-way admixed populations, removing one population at a time (other than Papuan) from the 18-population scaffold tree (Fig. 1). Values are means ± standard errors over the 17 different perturbed scaffolds. Austronesian ancestry refers to splits from the Ami and Atayal branches and their common ancestor, while Papuan support only includes splits from the Papuan branch. The results are very similar to those obtained with the original scaffold (Supplementary Table 4). Note that the branch support values are over 100 replicates, while the mixture proportions are point-estimates using all data rather than bootstraps.

Supplementary Table 11. Consistency of mixture parameters for three-way admixed populations on 17-population alternative scaffolds

E. Indonesian / Oceanian admixed population	Percent bootstrap reps with Branch 3 = H'tin, Plang, Wa	Branch 3 ancestry (Austro-Asiatic)	Branch 1 ancestry (Austronesian)
Manggarai Ngada	100 ± 1%	26 ± 2%	34 ± 2%
Manggarai Rampasasa	96 ± 15%	37 ± 2%	34 ± 2%
Toraja	99 ± 4%	12 ± 1%	72 ± 1%
W. Indonesian admixed population	Percent bootstrap reps with Branch 3 = H'tin, Plang, Wa	Branch 3 ancestry (Austro-Asiatic)	Branch 1 ancestry (Austronesian)
Batak Toba	93 ± 12%	27 ± 2%	53 ± 2%
Bidayuh	100 ± 0%	54 ± 2%	40 ± 1%
Dayak	100 ± 0%	39 ± 2%	52 ± 1%
Javanese Jakarta	100 ± 0%	59 ± 2%	32 ± 2%
Javanese Java	100 ± 0%	60 ± 2%	31 ± 2%
Malay Indonesia	97 ± 9%	31 ± 1%	60 ± 1%
Malay Singapore	98 ± 7%	41 ± 3%	40 ± 2%
Sunda	100 ± 0%	57 ± 2%	33 ± 1%

Mixture parameters from *MixMapper* for three-way admixed populations, removing one population at a time (other than Papuan) from the 18-population scaffold tree (Fig. 1). Values are means ± standard errors over the 17 different perturbed scaffolds. The results are very similar to those obtained with the original scaffold (see Supplementary Table 5). Mixture proportions shown are re-optimized values (see Methods), using the 17-population trees in which the third ancestry component is Austro-Asiatic (H'tin, Plang, or Wa), which were 16 of 17 for Batak Toba and Manggarai Rampasasa and all 17 trees for the other populations. Note that the branch support values are over 100 replicates, while the mixture proportions are point-estimates using all data rather than bootstraps.

Supplementary Table 12. Admixture model selection for three-way admixed populations

E. Indonesian / Oceanian admixed population	Residual norm from 2-way fit	Residual norm from 3-way fit	Difference (95% CI)
Manggarai Ngada	27.0	22.7	(-1.4, 9.8)
Manggarai Rampasasa	31.2	25.1	(-1.4, 14.5)
Toraja	11.3	7.9	(-0.8, 7.0)
W. Indonesian admixed population	Residual norm from 2-way fit	Residual norm from 3-way fit	Difference (95% CI)
Batak Toba	22.2	16.5	(-5.5, 15.2)
Bidayuh	23.1	15.5	(-1.6, 16.5)
Dayak	32.8	11.1	(11.4, 28.1)
Javanese Jakarta	34.3	15.3	(12.4, 23.8)
Javanese Java	32.8	15.0	(10.8, 24.0)
Malay Indonesia	18.8	10.1	(0.8, 14.9)
Malay Singapore	38.8	27.0	(0.6, 21.1)
Sunda	39.1	16.8	(15.8, 27.8)

Quality of fit for alternative models for three-way admixed populations. Shown are the median norms of the vectors of residual errors for all pairwise distances $f_2(C, X)$ (see Methods for details), along with 95% confidence intervals for the differences (all multiplied by 10^6). Smaller norms indicate more accurate model fits.

Supplementary Table 13. Two-way mixture fits for East and Mainland Southeast Asian populations

Admixed population	Mixing branch 1 + branch 2	% reps	Branch 1 ancestry
Chinese Singapore	(Ami,Atayal,Jiamao) + Karitiana	56%	98–99%
	(Ami,Atayal,Jiamao) + Naxi	21%	85–93%
	(Ami,Atayal,Jiamao) + Surui	15%	98–100%
Han Hakka	(Ami,Atayal,Jiamao) + Naxi	75%	83–91%
	(Ami,Atayal,Jiamao) + She	9%	58–89%
Han Minnan	(Ami,Atayal,Jiamao) + Naxi	63%	84–91%
	(Ami,Atayal,Jiamao) + Surui	13%	99–99%
	(Ami,Atayal,Jiamao) + Karitiana	13%	99–99%
	(Ami,Atayal,Jiamao) + She	8%	60–88%
Hmong China	Hmong Thailand + Jiamao	40%	71–89%
	Hmong Thailand + (Ami,Atayal,Jiamao)	34%	57–74%
	Hmong Thailand + She	20%	56–80%
Jinuo	(H'tin,Plang,Wa) + Yi	16%	77–91%
	(Naxi,Yi) + Wa	12%	52–80%
	(Karitiana,Mandenka,Naxi,Papuan,Surui,Yi,Yoruba,root) + Wa	11%	65–88%
	(H'tin,Plang,Wa) + (Naxi,Yi)	8%	41–83%
	(H'tin,Plang,Wa) + Hmong Thailand	7%	82–97%
	(H'tin,Plang,Wa) + Papuan	7%	97–99%
	(H'tin,Plang,Wa) + Naxi	6%	74–93%
Karen	(H'tin,Plang,Wa) + Papuan	93%	92–98%
	(H'tin,Plang) + Papuan	7%	90–96%
Lawa	(H'tin,Plang) + Papuan	82%	93–98%
	(H'tin,Plang,Wa) + Papuan	5%	95–98%
	H'tin + Papuan	5%	93–98%
Mlabri	H'tin + Papuan	70%	86–97%
	H'tin + (Mandenka,Yoruba,root)	18%	85–95%
	H'tin + (Mandenka,Yoruba)	9%	92–98%
Mon	(H'tin,Plang,Wa) + (Mandenka,Yoruba,root)	90%	80–86%
Tai Khuen	Jiamao + H'tin	99%	65–75%
Tai Lue	Jiamao + H'tin	97%	68–81%
Tai Yong	Jiamao + H'tin	95%	66–76%
Tai Yuan	Jiamao + H'tin	86%	48–60%
	(Ami,Atayal,Jiamao) + H'tin	10%	56–66%
Yao	(Ami,Atayal,Jiamao) + Hmong Thailand	79%	60–86%
	Hmong Thailand + H'tin	6%	87–94%
	(Ami,Atayal,Jiamao,She) + Hmong Thailand	6%	81–89%
Zhuang	Jiamao + H'tin	99%	87–92%

Inferred sources of ancestry (with bootstrap support) and mixture proportions (95% confidence intervals) from *MixMapper* for East and Mainland Southeast Asian populations. Names with parentheses refer to the common ancestral branches of the specified nodes (see Fig. 1). Branch topologies are shown that occur for at least 5% of 500 bootstrap replicates. We see essentially no evidence of the four ancestry components found in Austronesian-speaking groups, aside from H'tin-related (Austro-Asiatic) ancestry in several populations. We note that some of the populations here may not truly be admixed, but we show all of the fits for completeness.

Supplementary Table 14. Formal test for numbers of sources of admixture

Test subset	<i>p</i> -value for 2 sources	<i>p</i> -value for 3 sources	<i>p</i> -value for 4 sources
Agta, Ati, Ayta, Ilocano, Iraya, Manobo	0.000	0.110	0.156
Alorese, Kambera, Lamaholot, Lembata	0.000	0.486	0.428
Alorese, Kambera, Lamaholot, Lembata, Manggarai Ngada, Manggarai Rampasasa	0.000	0.000	0.366
Bidayuh, Dayak, Javanese Jakarta, Javanese Java, Mentawai, Sunda	0.000	0.000	0.068
Bidayuh, Dayak, Javanese Jakarta	0.000	0.018	NA

We applied a formal test based on f_4 statistics, as described in refs. (33) and (34), to estimate how many sources of admixture are necessary to explain the observed relationships among a collection of admixed populations. Briefly, we estimate the rank of a matrix of values $f_4(A, B; C, D)$, where A and B are populations in a test set and C and D are populations in a reference set. To remove trivially linearly dependent rows and columns, we fix A and C to be the first populations in each list (without loss of generality) and let B and D vary. In order to maximize sensitivity for separate sources of Asian ancestry, we used a reference set consisting of Yoruba as the fixed outgroup C and 31 East and Southeast Asian populations as the other references D . We used a p -value threshold of 0.05; a score below this threshold implies that at least that many sources are necessary to explain the relationships among the test set. In bold are the maximal significant values, indicating the estimated number of sources for each set.

Supplementary Table 15. Mixture fits for Austronesian-speaking populations with no Taiwanese in the scaffold tree

Admixed population	Mixing branch 1 + branch 2	% reps	Branch 1 ancestry
Agta	Jiamao + Papuan	96%	51–62%
Alorese	Papuan + H'tin	81%	55–62%
	Papuan + (H'tin,Plang)	9%	55–62%
Ami	Jiamao + H'tin	43%	85–95%
	Jiamao + Karitiana	36%	98–99%
	Jiamao + Surui	15%	98–99%
Atayal	Jiamao + (Karitiana,Surui)	30%	88–97%
	Jiamao + Papuan	14%	93–99%
	Jiamao + (Mandenka,Papuan,Yoruba,root)	12%	88–97%
	Jiamao + (Karitiana,Mandenka,Papuan,Surui,Yoruba,root)	10%	71–88%
	Jiamao + (Mandenka,Yoruba,root)	8%	94–98%
	Jiamao + H'tin	7%	78–95%
	Jiamao + Surui	7%	97–99%
Ati	Jiamao + Papuan	95%	50–59%
Ayta	Papuan + H'tin	92%	61–75%
	Papuan + Jiamao	5%	63–80%
Bidayuh	Manobo + H'tin	100%	30–42%
Dayak	Manobo + H'tin	100%	46–59%
Iraya	Jiamao + Papuan	77%	68–80%
	Jiamao + (Mandenka,Papuan,Yoruba,root)	21%	57–71%
JavaneseJakarta	Manobo + H'tin	100%	34–46%
Kambera	(H'tin,Plang,Wa) + Papuan	42%	71–76%
	Jiamao + Papuan	34%	67–74%
	(HmongThailand,Jiamao,Miao,She) + Papuan	21%	69–75%
Mamanwa	Jiamao + Papuan	76%	50–60%
	H'tin + Papuan	12%	52–59%
	(H'tin,Plang,Wa) + Papuan	9%	53–64%
Manobo	Jiamao + Papuan	100%	77–84%
Sunda	Manobo + H'tin	100%	40–51%

Inferred sources of ancestry (with bootstrap support) and mixture proportions (95% confidence intervals) from *MixMapper* for selected Austronesian-speaking populations, using a 16-population scaffold tree formed by removing Ami and Atayal from the original scaffold (i.e., Miao, She, Jiamao, Lahu, Wa, Yi, Naxi, Hmong, Plang, H'tin, Palaung, Karitiana, Surui, Papuan, Mandenka, and Yoruba). Names with parentheses refer to the common ancestral branches of the specified nodes (see Fig. 1). Branch topologies are shown that occur for at least 5% of 500 bootstrap replicates. We report admixture fits for Ami and Atayal as test populations, as well as all other Austronesian-speaking populations with no negative f_3 statistics (Supplementary Table 3) and selected others to fill in geographic coverage gaps. For both Ami and Atayal, more than half of the bootstrap replicates yield fits with 90% or more Jiamao ancestry and a very small proportion of a seemingly implausible second ancestry component (e.g., Native American). In our experience, such results indicate that the test populations should in fact be modeled as unadmixed relative to the scaffold, in this case adjacent to Jiamao (31). For other populations, meanwhile, the fits appear to be reasonable and are very similar (both in topology and mixture proportions) to those obtained with the original scaffold (with the difference that Jiamao is now the closest population to the previous location of the Taiwanese). Fits with Manobo reported as one mixing branch are three-way admixtures (proportions are not re-optimized).

Supplementary Table 16. Robustness of Austro-Asiatic ancestry with modified scaffolds

E. Indonesian / Oceanian admixed population	Percent bootstrap support with H'tin removed	Percent bootstrap support with H'tin and Plang removed
Manggarai Ngada	95%	16%
Manggarai Rampasasa	44%	0%
Toraja	84%	36%
W. Indonesian admixed population	Percent bootstrap support with H'tin removed	Percent bootstrap support with H'tin and Plang removed
Batak Toba	45%	24%
Bidayuh	100%	98%
Dayak	100%	93%
Javanese Jakarta	100%	100%
Javanese Java	100%	100%
Malay Indonesia	69%	29%
Malay Singapore	63%	31%
Sunda	100%	100%

Robustness of the Austro-Asiatic ancestry component from *MixMapper* for three-way admixed populations with either H'tin or H'tin and Plang removed from the 18-population scaffold tree. Shown are the percentages of bootstrap replicates (out of 500) assigning the third ancestry component in a three-way admixture model to an Austro-Asiatic branch in the scaffold (Plang or Wa in the first column and Wa in the second column). The fits on the reduced scaffolds are not as robust for the eastern Indonesian populations, while the lower confidences for Batak Toba and the Malay populations may be due to a small proportion of Indian ancestry (20, 25) that is picked up more often with fewer Austro-Asiatic references present.